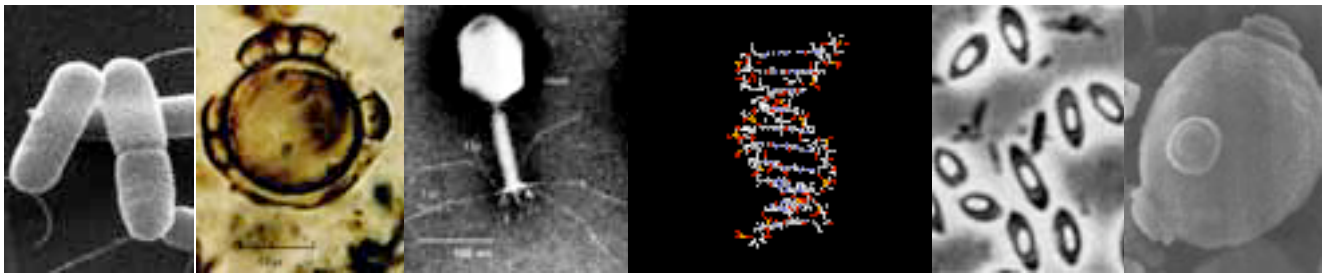


Microbial genetics

General Microbiology - Lecture 7

Cañada College - Fall 2008

Instructor: Tamas Torok, Ph.D.

Overview for today

- **Structure and function of the genetic material**
  - DNA replication
  - transcription and translation of genetic information
- **Regulation of gene expression**
  - induction and repression
  - operon model
- **Change of the genetic material**
  - mutation and recombination

Microbial diversity and evolution

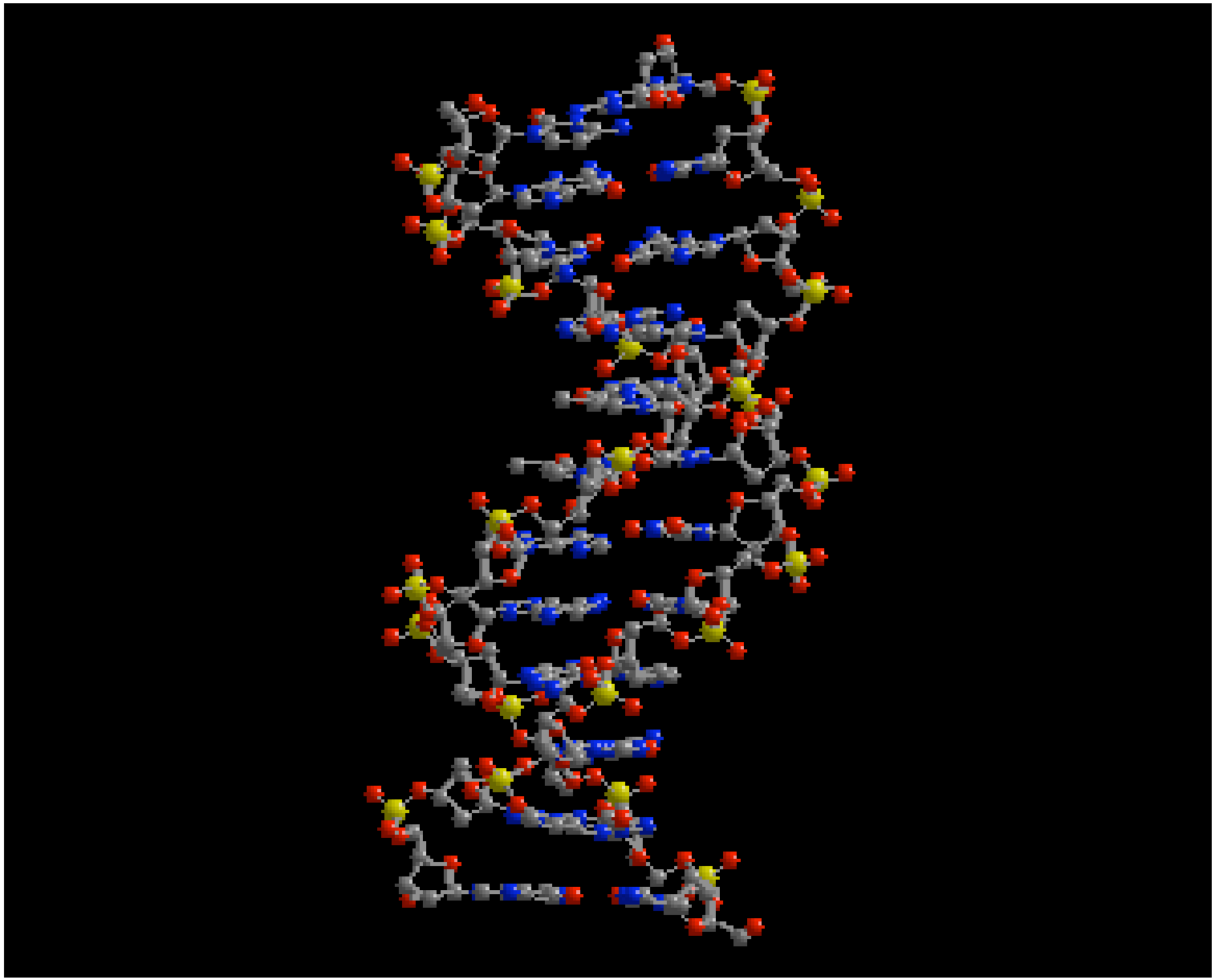
- **"...Microbial diversity includes the genetic composition of microorganisms, their environment or habitat where they are found, and their ecological or functional role within the ecosystem…"**

J. C. Hunter-Cevera, 2000

- **Evolution is the long-term process of natural selection acting on diversity to ensure the survival of the fittest**

Genetics

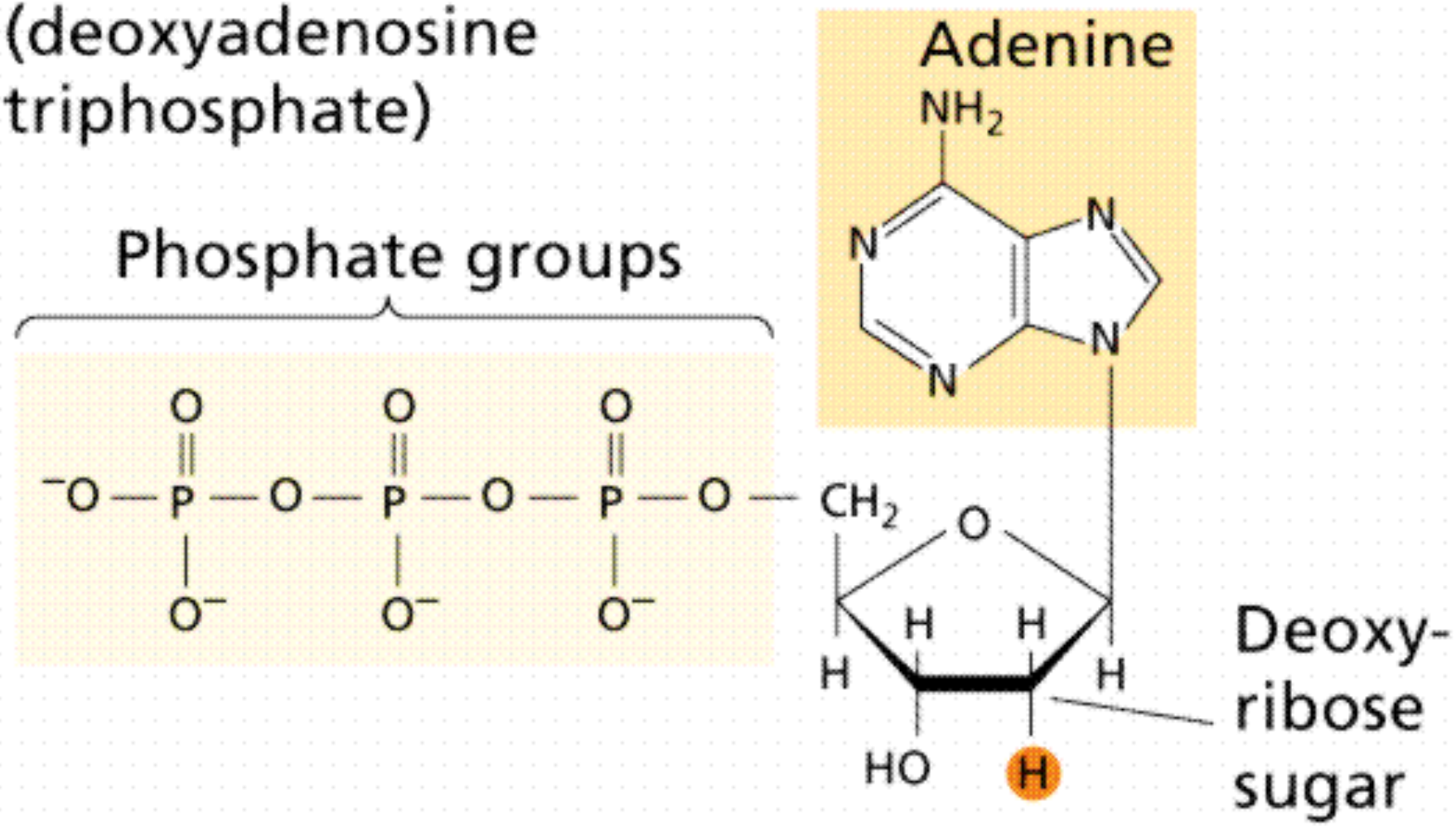
- Study of heredity - study of the genotype
- Information is stored in sequences of nucleic acids
- Gene
  - a segment of the genetic information that codes for a structure or function
- Chromosome
  - organization of genetic information, including coding and “non-coding” sequences
- Genome
  - entirety of genetic information in a cell, including chromosomal and extra-chromosomal information



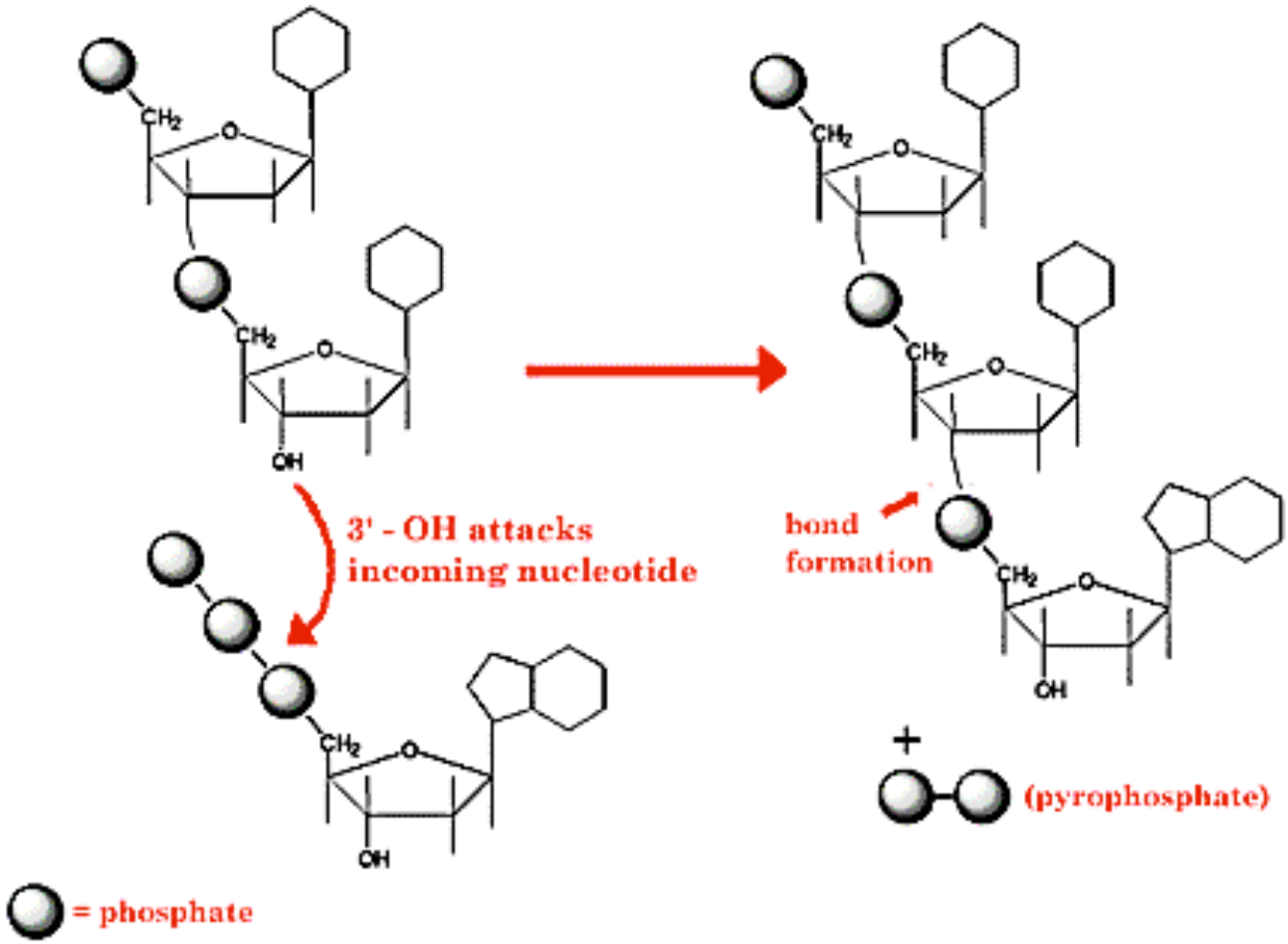
DNA structure and organization

- **DNA**
  - macromolecule composed of repeating monomers, ***nucleotides***
  - forms a double helix that is supercoiled
    - role of histones in Archaea and Eucarya
  - strands are ***complementary*** and ***antiparallel*** held together by hydrogen bonds
- **Genetic code**
  - genetic diversity is based on 4^n (n = genome size)
  - genetic code - set of rules of conversion

Deoxy-ATP
(deoxyadenosine
triphosphate)



Polymerization reaction

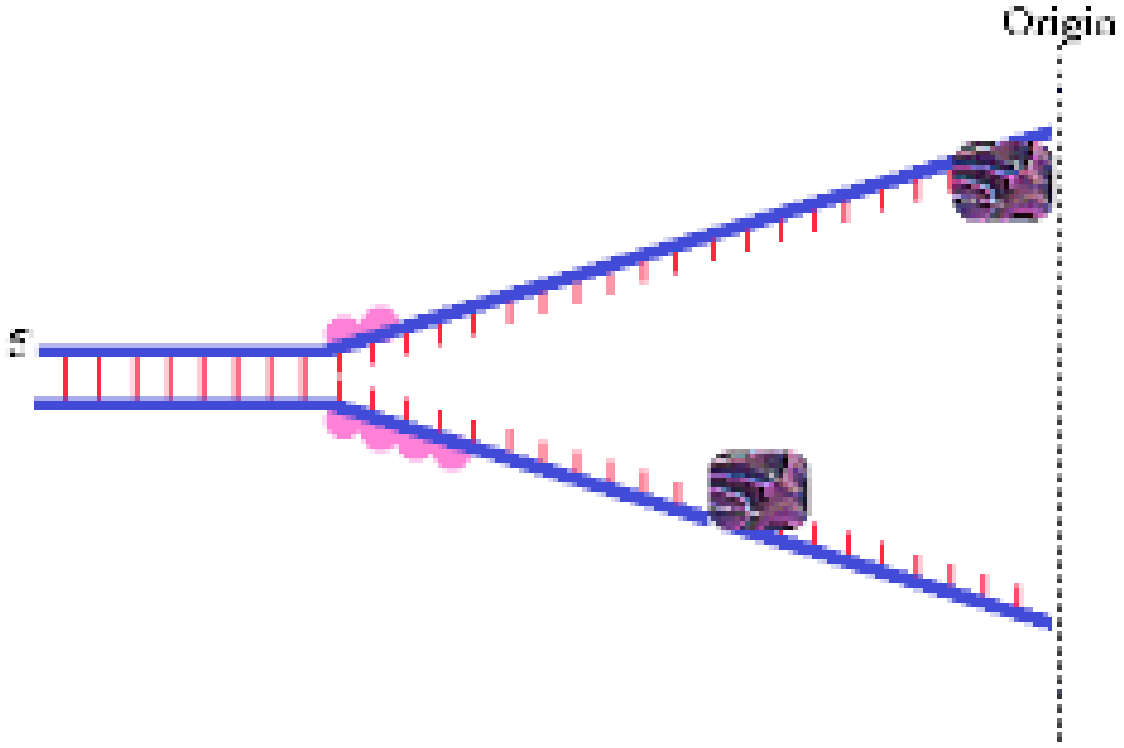


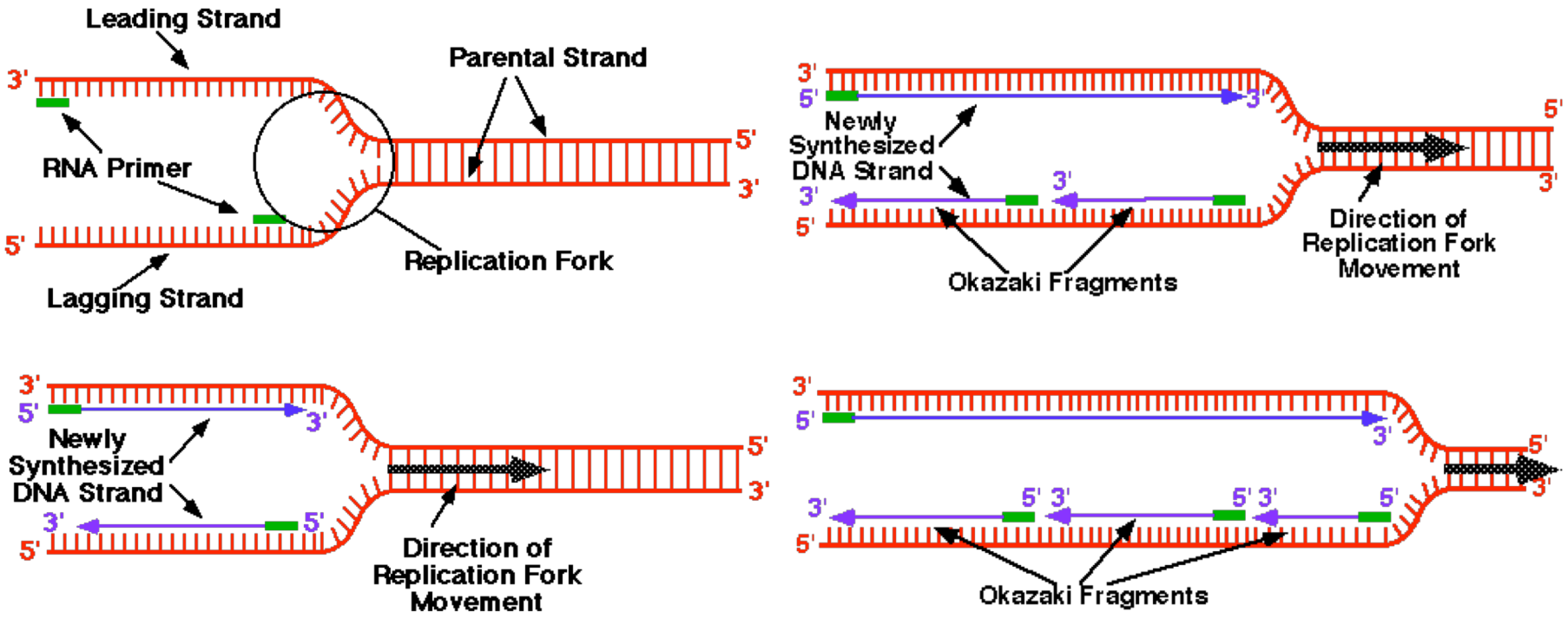
DNA replication

- ***Semi-conservative*** **replication**
  - **each new double stranded DNA molecule contains one original and one newly synthesized strand**
- **Highly conserved process that requires timing and a large number of factors and proteins for**
  - **unwinding**
  - **synthesis with proof-reading capability**
  - **ligation**
  - **rewinding**

DNA replication (cont.)

- **At the replication fork DNA polymerases are adding nucleotides to the 3' end of a small molecule ("oligo" or "primer")**
  - ***"leading strand"*** **- synthesis is proceeding continuously in the direction of the opening replication fork**
  - ***"lagging strand"*** **- on the opposite strand DNA synthesis is discontinuous; it proceeds *away* from the opening replication fork**
    - **Okazaki fragments are produced that need to be ligated together**

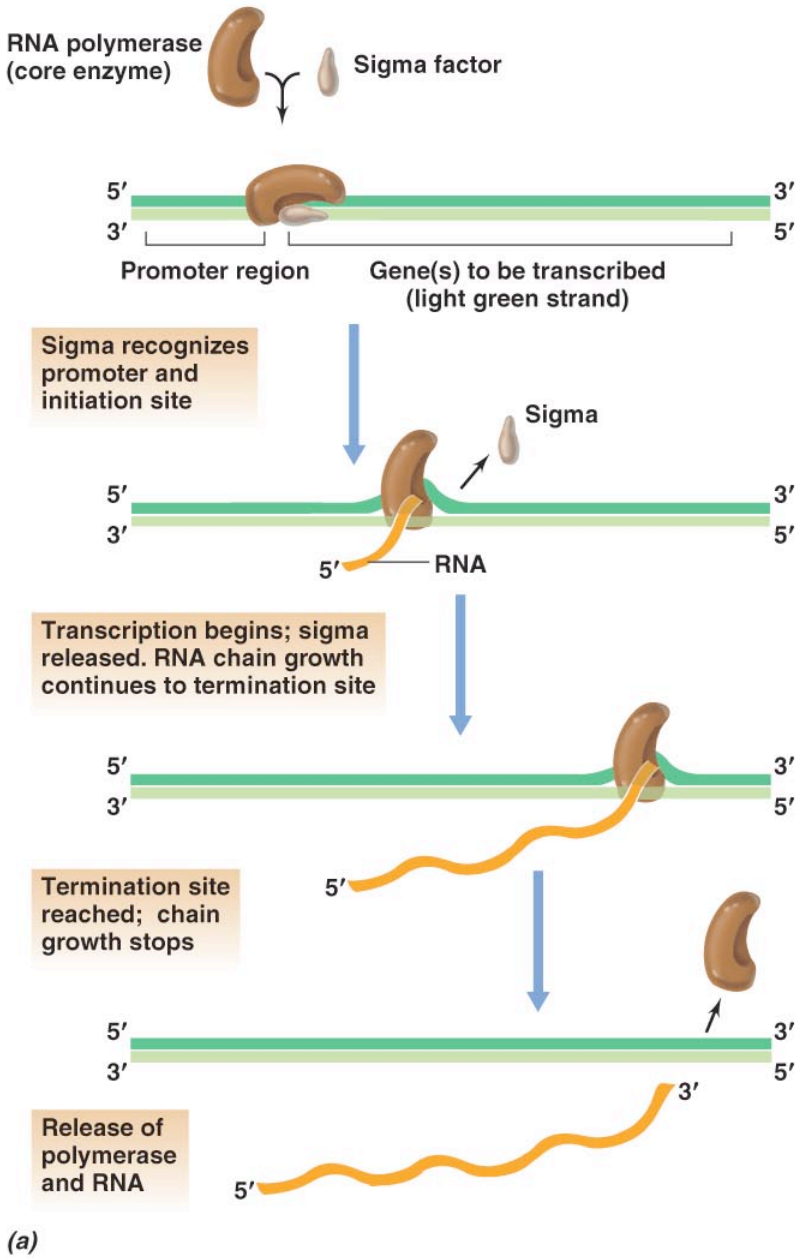

Origin
5'


Leading Strand
Parental Strand
3'
5'
RNA Primer
Replication Fork
Lagging Strand
Newly
Synthesized
DNA Strand
Okazaki Fragments
Direction of
Replication Fork
Movement

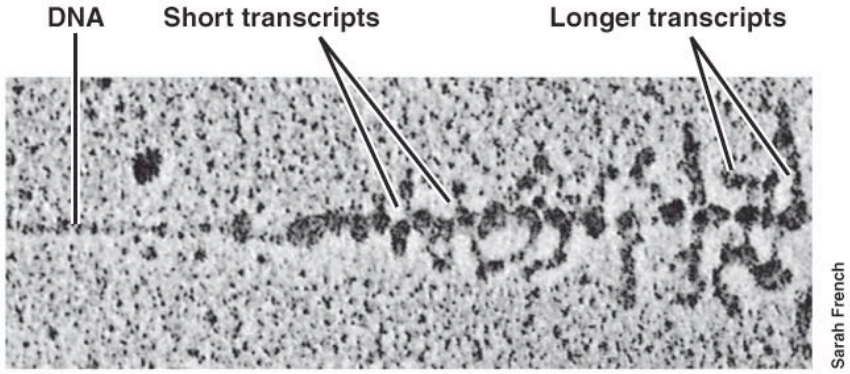
Transcription of DNA information

- **During RNA synthesis DNA template information is transcribed to a complementary RNA strand**
  - **RNA polymerase binds to DNA promoter region**
  - **transcribes only one strand information in 5'→3' direction**
  - **RNA synthesis ends at terminator**
  - **RNA processing in eukaryotes (splicing out introns)**
- **Classes of RNA**
  - **rRNA - integral part of ribosomes**
  - **mRNA - carries DNA information to ribosomes**
  - **tRNA - activates and transports amino acids to ribosomes**
  - **ribozyme**
  - **small, interference RNA molecules (iRNA)**

RNA synthesis



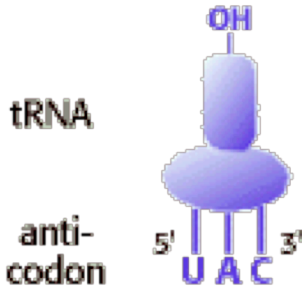
(a)



(b)

Translation of DNA information

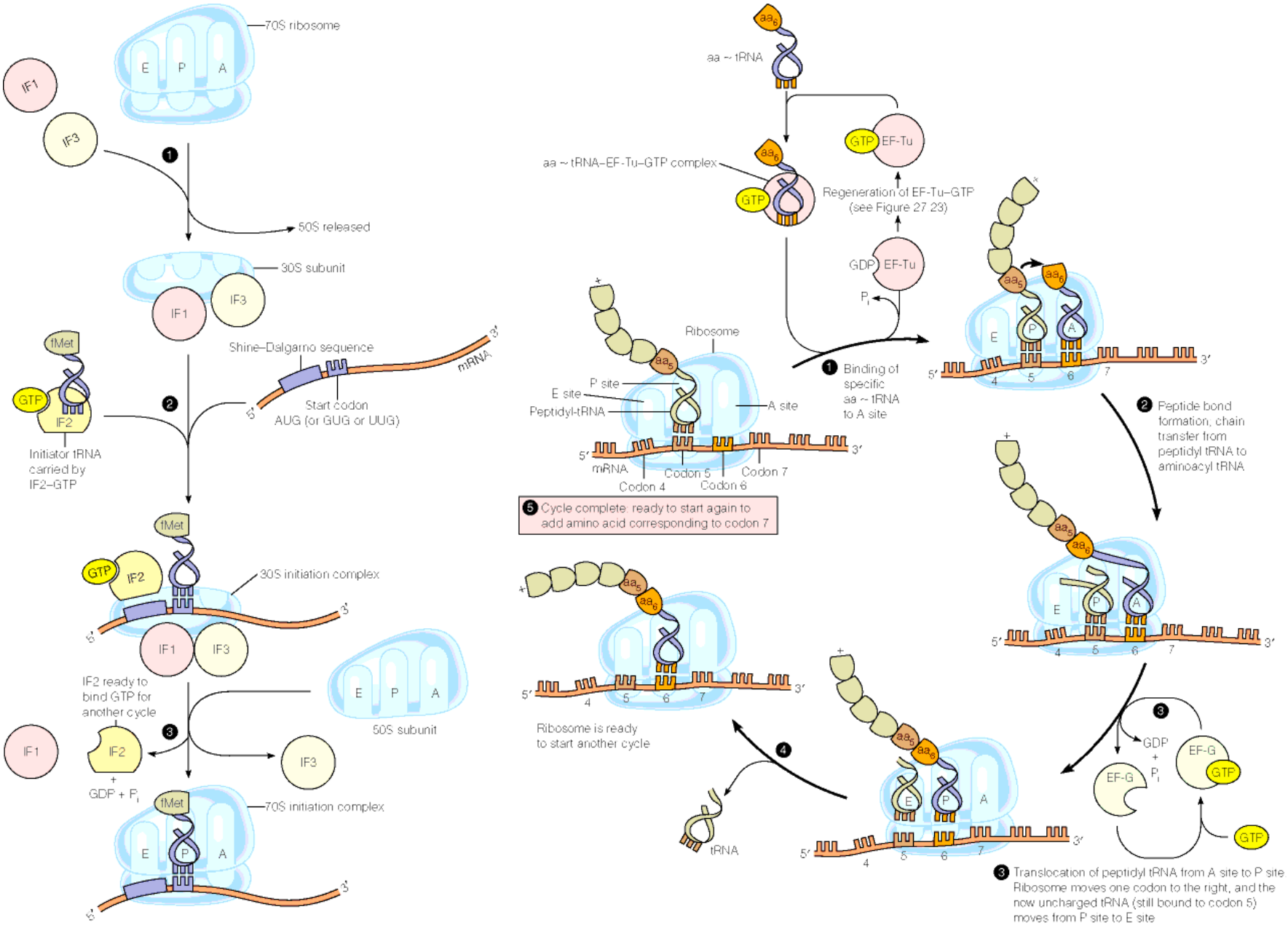
- **Protein synthesis occurs on the active surface of ribosomes**
- **mRNA information is contained in codon triplets**
  - **genetic code**
  - **genetic code is degenerate (64 codons vs. 22 AA)**
  - **sense vs. nonsense codons**
    - **AUG - start codon, codes for methionine in bacteria**
    - **tRNA w/anticodon**



Second Base

First Base	U		C		A		G		Third Base
U	UUU	phe	UCU	ser	UAU	tyr	UGU	cys	U
	UUC	phe	UCC	ser	UAC	tyr	UGC	cys	C
	UUA	leu	UCA	ser	UAA	STOP	UGA	STOP	A
	UUG	leu	UCG	ser	UAG	STOP	UGG	trp	G
C	CUU	leu	CCU	pro	CAU	his	CGU	arg	U
	CUC	leu	CCC	pro	CAC	his	CGC	arg	C
	CUA	leu	CCA	pro	CAA	gln	CGA	arg	A
	CUG	leu	CCG	pro	CAG	gln	CGG	arg	G
A	AUU	ile	ACU	thr	AAU	asn	AGU	ser	U
	AUC	ile	ACC	thr	AAC	asn	AGC	ser	C
	AUA	ile	ACA	thr	AAA	lys	AGA	arg	A
	AUG	met	ACG	thr	AAG	lys	AGG	arg	G
G	GUU	val	GCU	ala	GAU	asp	GGU	gly	U
	GUC	val	GCC	ala	GAC	asp	GGC	gly	C
	GUA	val	GCA	ala	GAA	glu	GGA	gly	A
	GUG	val	GCG	ala	GAG	glu	GGG	gly	G

	Amino Acid
1	Alanine
2	Arginine
3	Aspargine
4	Aspartic Acid
5	Cysteine
6	Glutamine
7	Glutamic Acid
8	Glycine
9	Histamine
10	Isoleucine
11	Leucine
12	Lysine
13	Methionine
14	Phenylalanine
15	Proline
16	Serine
17	Threonine
18	Tryptophan
19	Tyrosine
20	Valine
21	Selenocysteine
22	Pyrrolysine


70S ribosome
E P A
IF1
IF3
50S released
30S subunit
IF1
IF3
fMet
GTP
IF2
Initiator tRNA carried by IF2–GTP
Shine–Dalgarno sequence
mRNA
Start codon AUG (or GUG or UUG)
fMet
GTP
IF2
30S initiation complex
IF1
IF3
IF2 ready to bind GTP for another cycle
IF1
IF2
GDP + Pi
IF3
50S subunit
fMet
70S initiation complex
aa ~ tRNA
GTP EF-Tu
aa ~ tRNA–EF-Tu–GTP complex
Regeneration of EF-Tu–GTP (see Figure 27 23)
GDP EF-Tu
Pi
Ribosome
P site
E site
Peptidyl-tRNA
A site
mRNA
Codon 4
Codon 5
Codon 6
Codon 7
1 Binding of specific aa ~ tRNA to A site
2 Peptide bond formation, chain transfer from peptidyl tRNA to aminoacyl tRNA
3
GDP + Pi
EF-G
EF-G
GTP
GTP
3 Translocation of peptidyl tRNA from A site to P site Ribosome moves one codon to the right, and the now uncharged tRNA (still bound to codon 5) moves from P site to E site
4
tRNA
Ribosome is ready to start another cycle
5 Cycle complete: ready to start again to add amino acid corresponding to codon 7

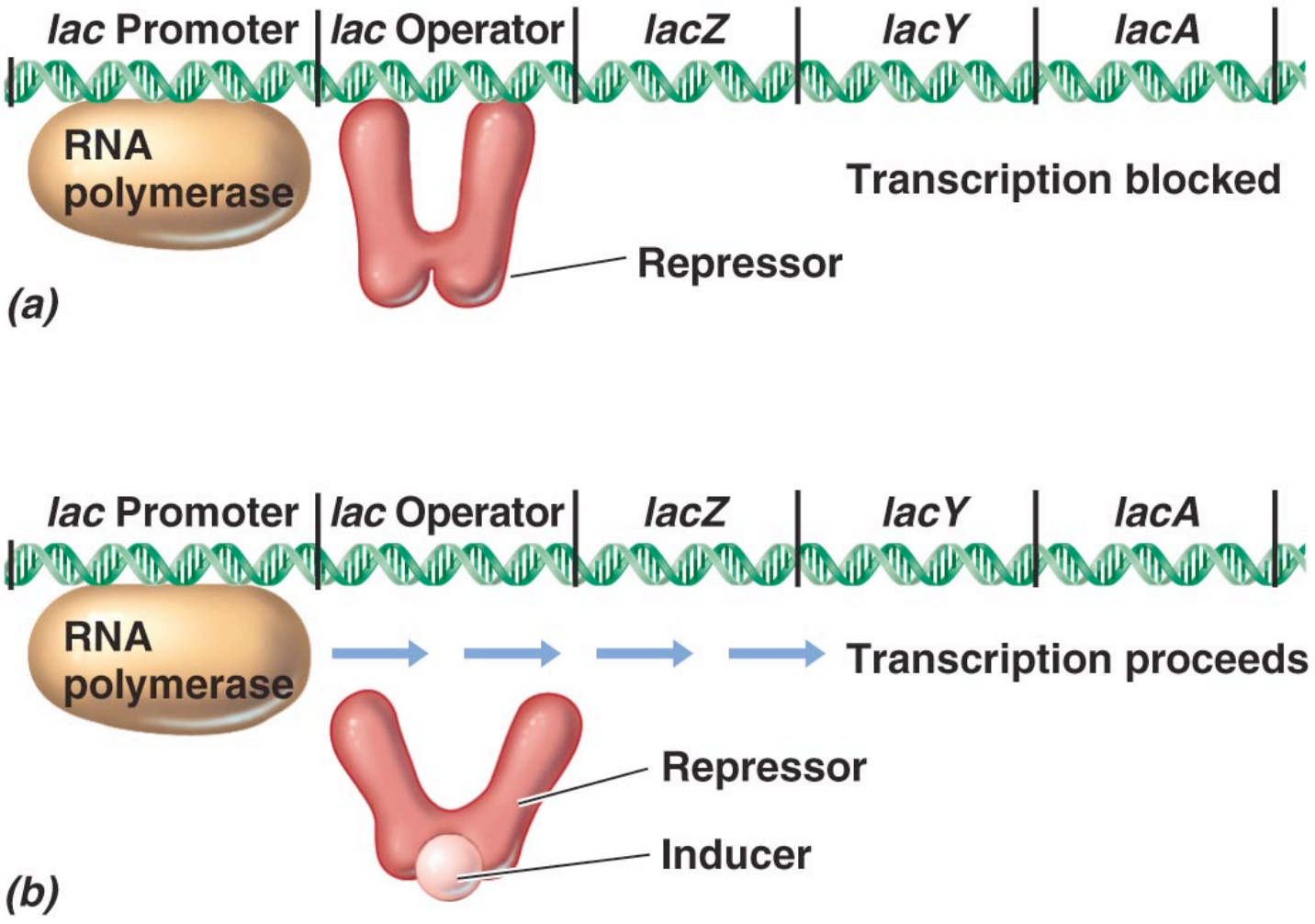
Regulation of gene expression

- 60-80% of gene products are constitutive
- Repression
  - inhibition of gene expression (and enzyme synthesis)
  - repressor molecule is usually a product of the biochemical pathway
- Induction
  - process of turning on gene transcription
  - inducer molecule is usually a substrate molecule or an ecological factor
- Post-translational regulation in eukaryotes

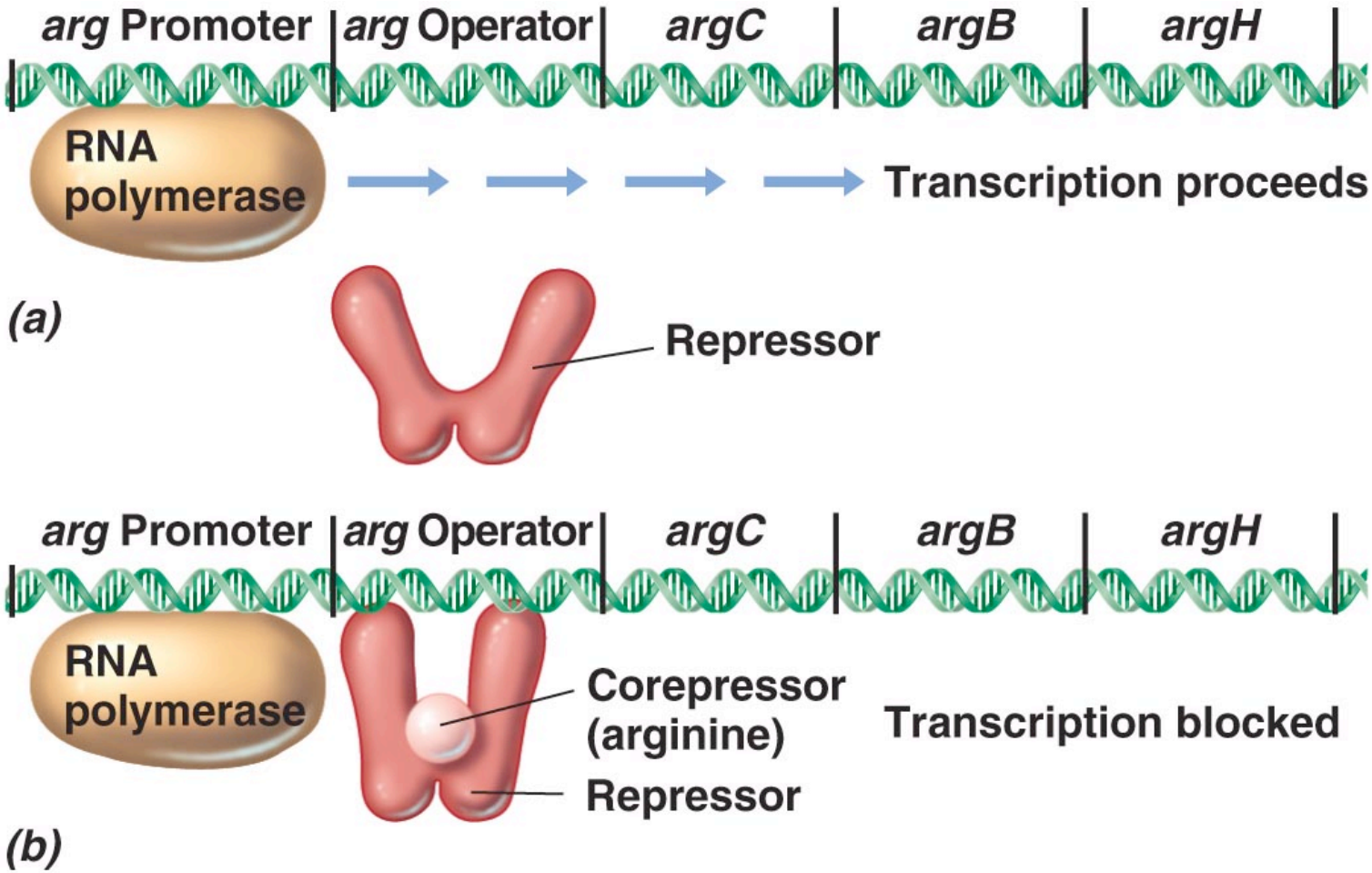
Operon model

- **Jacob-Monod model (1961) of protein synthesis in *E. coli***
- **β-galactosidase induction in the presence of lactose**
- **Structural and regulator genes and adjoining control regions are organized in an *operon***

Enzyme induction



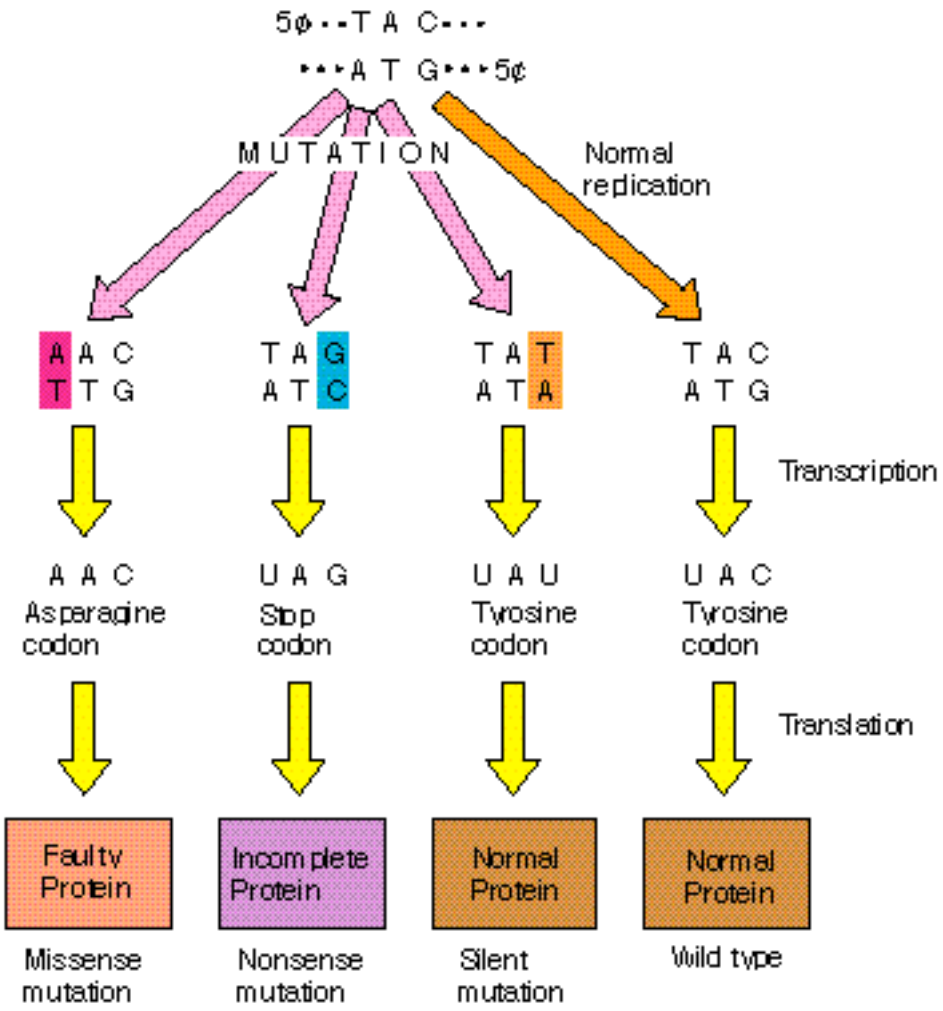
Enzyme repression



Mutation

- **Mutation is an inherited change in the genetic material**
  - **DNA polymerases work with high fidelity (one mistake in every 10^{10} bases read/synthesized)**
  - **environmental factors cause DNA damage**
  - **unless one of the many repair mechanisms fixes the damage, mutation results**
- **Base substitution (point mutation)**
  - **missense mutation - change in AA sequence**
  - **nonsense mutation results in a stop codon that terminates protein synthesis prematurely**
- **Insertion/deletion may cause frameshift mutation**
- **Spontaneous mutation vs. the effect of mutagens**

Influence of mutation

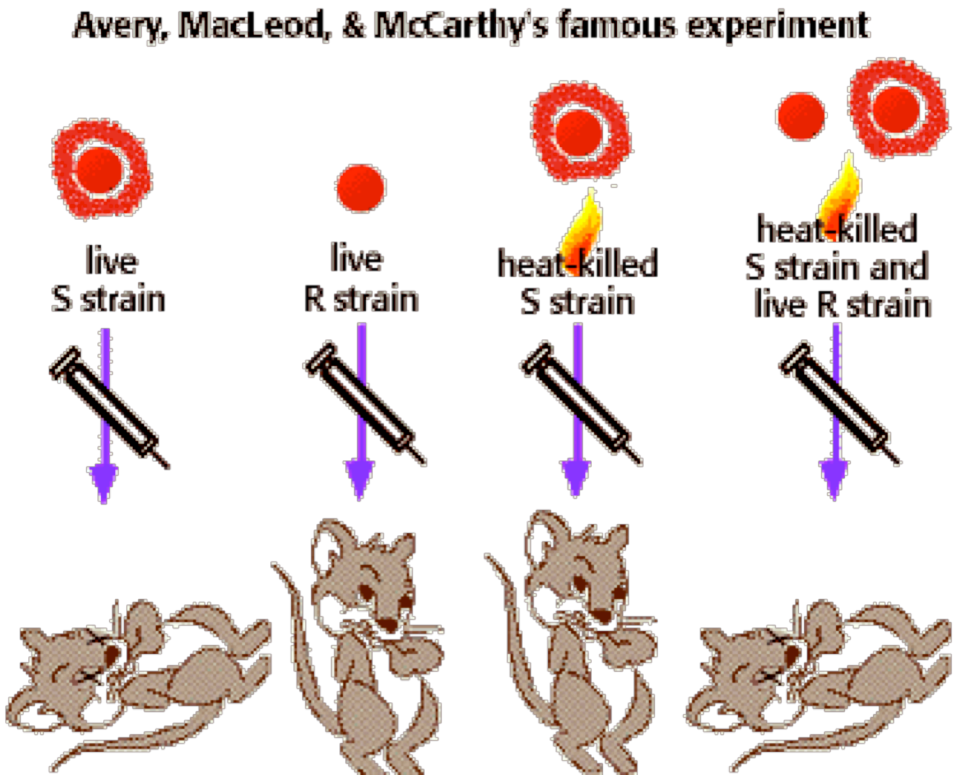


Genetic transfer

- **Genetic recombination**
  - exchange of genetic material that results in new genetic combinations
  - vertical gene transfer
    - genetic information is passed on to the offspring
  - horizontal gene transfer
    - real-time gene transfer from donor to recipient
    - major role in evolution
  - in eukaryotes sex is the check
  - Bacteria and Archaea have no “true” sex

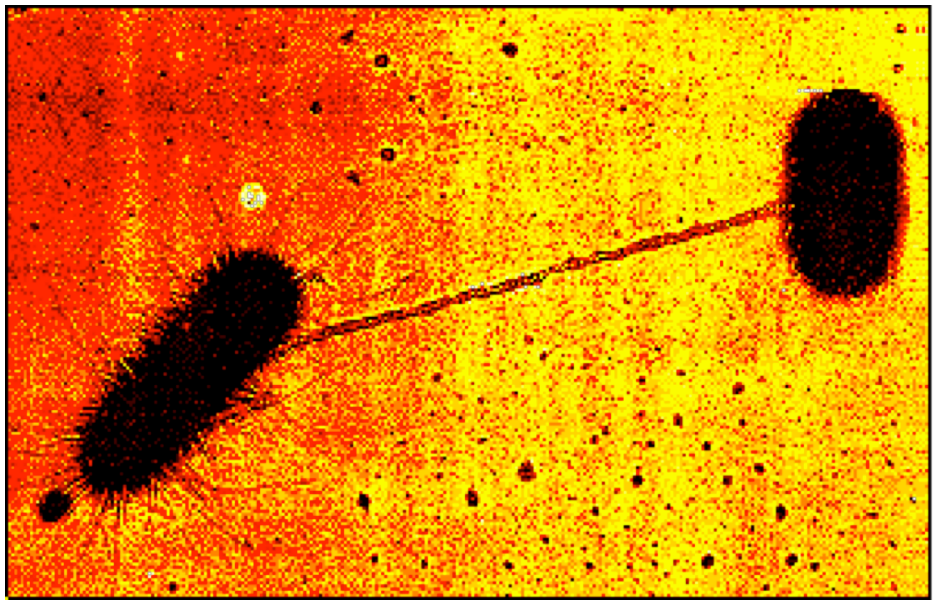
Transformation of bacteria

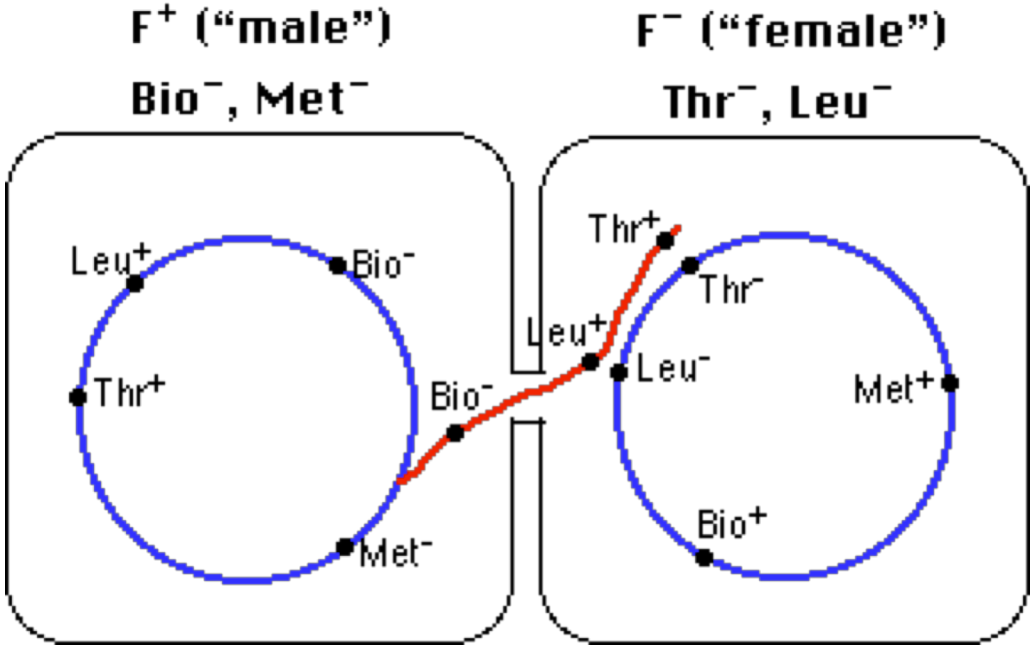
- **Transfer of a "naked" piece of DNA**



Conjugation in bacteria

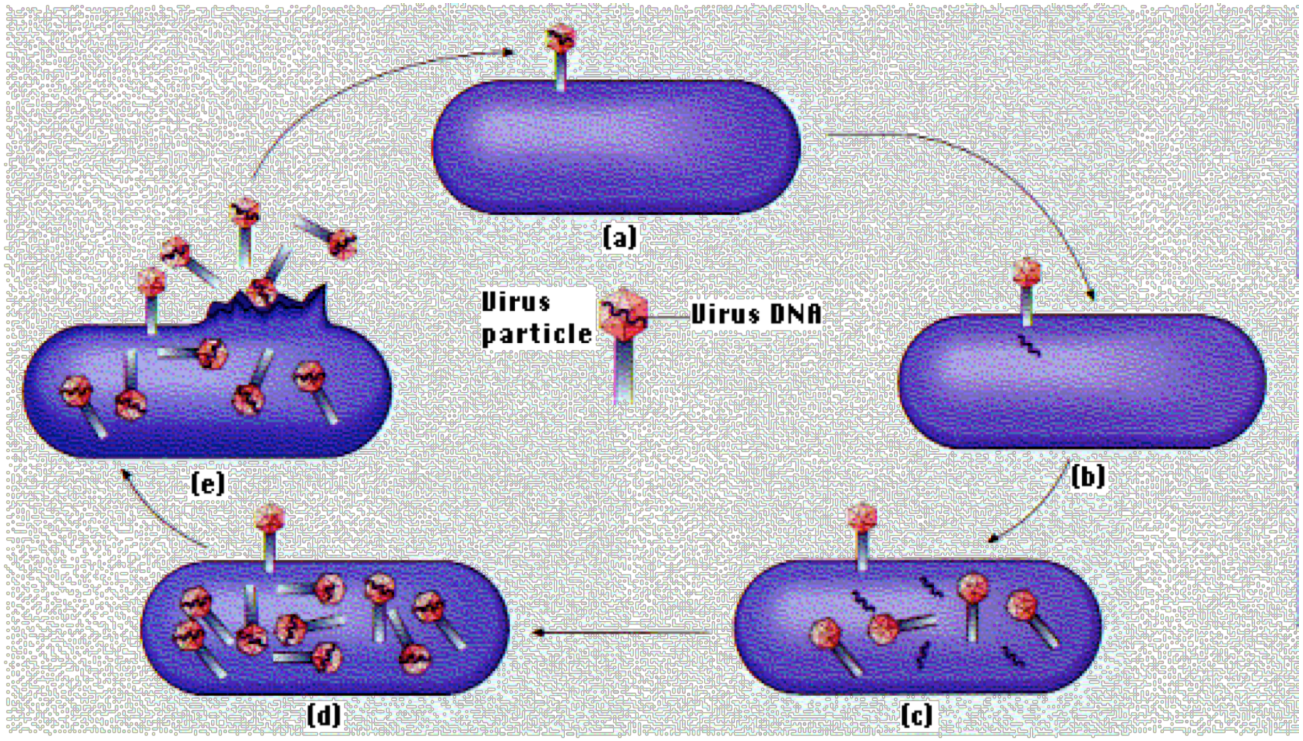
- **Cell-to-cell direct contact is required**
  - **sex pili make the connection in Gram (-) bacteria**
  - **sticky surface molecules in Gram (+) bacteria**
- **F factor in *E. coli***
  - **donor (F^+ cell) plasmid transforms recipient F^- cell**
  - **if plasmid integrates into the chromosome of the recipient cell, it becomes a HFr cell**
  - **conjugation between a HFr and F^- cells may result in the entire parental chromosome being transferred**




F+ ("male")
Bio−, Met−
F− ("female")
Thr−, Leu−
Leu+
Bio−
Thr+
Met−
Thr+
Thr−
Leu+
Leu−
Bio−
Met+
Bio+

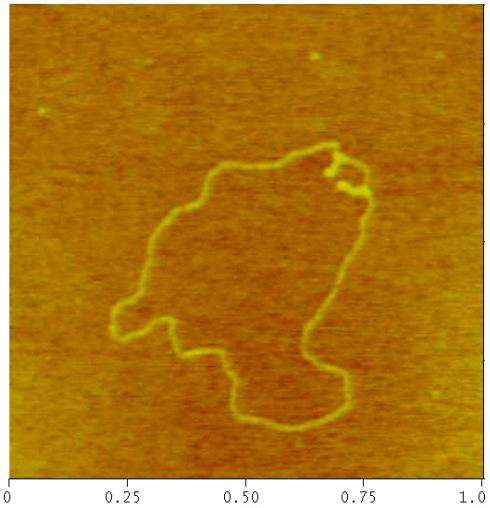
Transduction in bacteria

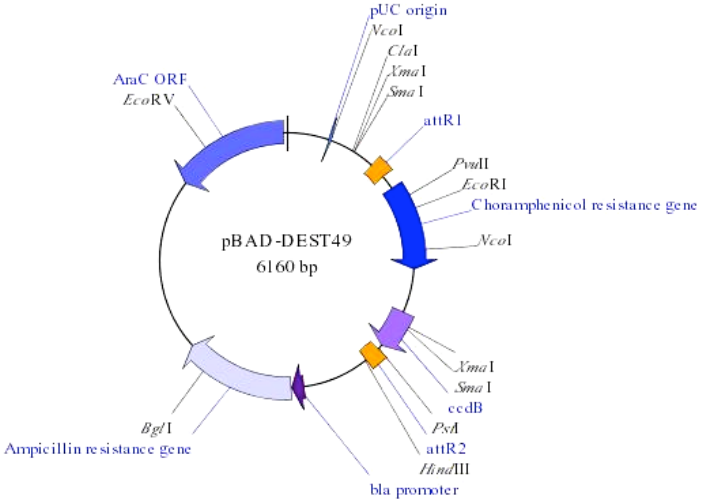
- **Donor cell DNA is transferred into the recipient cell via a bacterial virus (bacteriophage)**



Mobile genetic elements

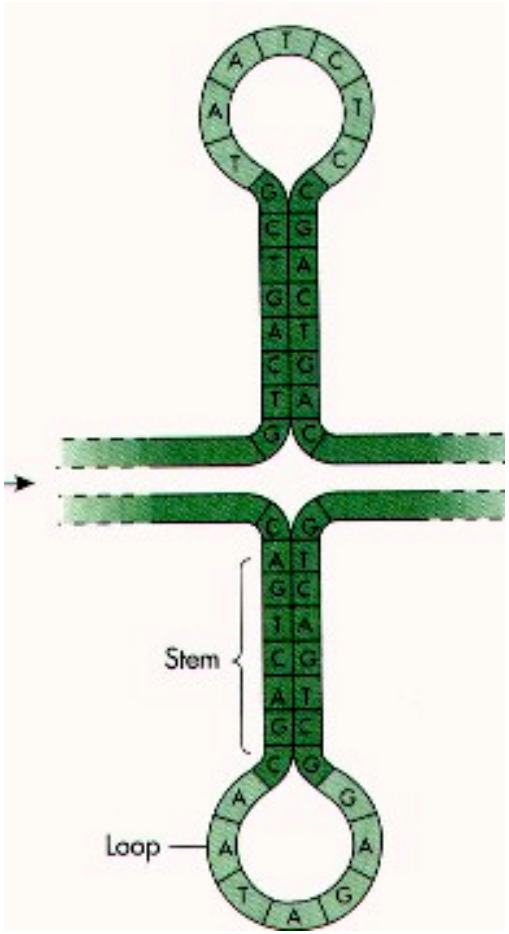
- **Plasmid**
  - **self-replicating genetic element**
    - **own origin of replication**
    - **copy number may vary**
    - **may carry genes for resistance against toxic substances**
    - **responsible for the spread of antibiotic resistance**





Mobile genetic elements

- **Transposon**
  - **“jumping” DNA fragment**
  - **integration into chromosome via inverted repeat sequences (palindromes)**



Evolutionary importance of genetic recombination

- Genomes can be either clonal or mosaic type
  - genetic diversity
    - clonal type - periodic selection is the cohesive evolutionary force
    - mosaic type - conserved core with genomic islands with different evolutionary histories
- Lateral transfer of mobile genetic elements (plasmids, transposons, operons, phages)
- Integration, independent expression, or elimination of foreign genetic information